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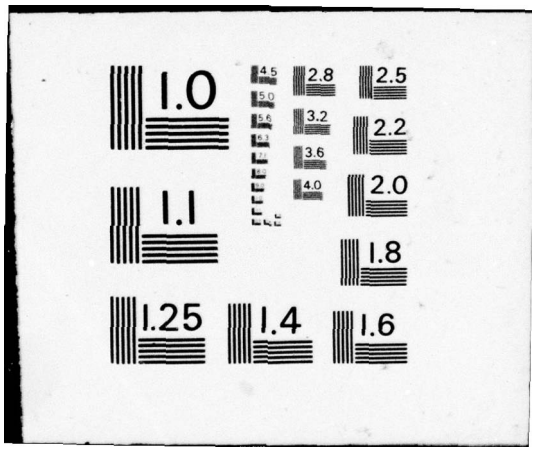
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CHEMICAL ANALYSIS OF SULFONES USED FOR THE TREATMENT OF MALARIA

Annual Report No. 2

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By
JOHN H. PETERS

September 1976

Supported by

U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
WASHINGTON, D.C. 20314

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Attempts were continued to identify minor contaminants previously resolved from ethanolic extracts of dapsone (DDS) tablets by thin-layer and high-pressure liquid chromatography. By comparative mass spectrometry, one of the contaminants was identified as 3,4'-diaminodiphenyl sulfone. Mass spectrometry also revealed that another contaminant exhibited the characteristics of a sulfone and of a parent ion, suggesting that it is a dimer of DDS. This contaminant was not identified with certainty, however. As with other sulfone contaminants of DDS tablets, no differences were found in the amounts of 3,4'-diaminodiphenyl sulfone or of the probable DDS dimer in

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20 ABSTRACT (Continued)

samples of lots of DDS tablets that had been returned from overseas and in samples of the same lots that had been stored in U.S. depots.

An additional unknown contaminant, originally found by thin-layer chromatography, had a very long retention time on high-pressure liquid chromatography. This material was isolated, and it exhibited absorption characteristics different from those typical of sulfones. Also, in this material no volatile compound typical of sulfones was found on direct-probe mass spectrometric analysis. This compound has not been identified.

Initial studies on the photodecomposition of DDS in organic solvents revealed that the rate of disappearance depended on concentration, the solvent used, and the method of analysis. Measurements of the loss of fluorescence of DDS proved to be unreliable at high DDS concentrations compared with determinations of DDS losses by high-pressure liquid chromatography. The high rate of loss of DDS in ethylene dichloride was shown to result from effects on DDS by products of the irradiation of this solvent as well as by direct photodecomposition of DDS. Preirradiation of ethanol did not cause photodecomposition of DDS dissolved in this solvent. Photodecomposition rates of pure DDS in ethanol and of ethanolic extracts of DDS tablets were found to be essentially the same. No relationship between the history of storage of the tablets and the rate of photodecomposition was found. Use of ethanol that had been saturated with oxygen or nitrogen for dissolving the DDS had no significant influence on the photodecomposition rate of DDS.

High-pressure liquid chromatography of irradiated ethanolic solutions of DDS revealed several new peaks not found previously as DDS contaminants in tablets. The major peak was identified as 2-amino-4'-nitrodiphenyl sulfone by its chromatographic properties and mass spectrophotometric fragmentation pattern. Other peaks were not identified. 2-Amino-4'-nitrodiphenyl sulfone accounted for between 25 and 50% of the DDS lost during irradiation.

Initial studies showed that the rate of photodecomposition of irradiated DDS in water was approximately ten times faster than that previously observed in ethanol. Also, two peaks were found with longer retention times during high-pressure liquid chromatography than had been observed previously. The major peak was identified by isolation and mass spectrometry as 4-amino-4'-(2-phenylhydrazino)diphenyl sulfone. On mass spectrometry, the minor one exhibited the same parent ion as the major peak and was considered to be an isomer of the above compound.

Rates of disappearance ($T\frac{1}{2}$) of DDS in water were essentially the same whether pure DDS or aqueous or ethanolic extracts of DDS tablets were used. $T\frac{1}{2}$ values ranged from 0.23 to 0.58 hr at 10 μg DDS/ml, from 1.6 to 2.5 hr at 100 μg DDS/ml, and from 3.2 to 4.6 hr at 170 μg DDS/ml. Tests of the effects of presaturation with oxygen or nitrogen of the water used to dissolve the DDS were performed. The finding of significantly shorter $T\frac{1}{2}$ values for pure DDS and DDS from tablets dissolved in water purged with nitrogen suggested that the absence of oxygen in this water resulted in more rapid photodecomposition.

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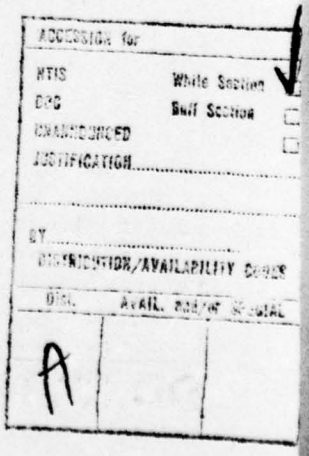

SUMMARY

Attempts were continued to identify minor contaminants previously resolved from ethanolic extracts of dapsone (DDS) tablets by thin-layer and high-pressure liquid chromatography. By comparative mass spectrometry, one of the contaminants was identified as 3,4'-diaminodiphenyl sulfone. Mass spectrometry also revealed that another contaminant exhibited the characteristics of a sulfone and of a parent ion, suggesting that it is a dimer of DDS. This contaminant was not identified with certainty, however. As with other sulfone contaminants of DDS tablets, no differences were found in the amounts of 3,4'-diaminodiphenyl sulfone or of the probable DDS dimer in samples of lots of DDS tablets that had been returned from overseas and in samples of the same lots that had been stored in U.S. depots. An additional unknown contaminant, originally found by thin-layer chromatography, had a very long retention time on high-pressure liquid chromatography. This material was isolated, and it exhibited absorption characteristics different from those typical of sulfones. Also, in this material no volatile compound typical of sulfones was found on direct-probe mass spectrometric analysis. This compound has not been identified.

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TABLES (continued)

FIGURES

INTRODUCTION AND BACKGROUND

To determine whether a causal relationship existed between the occasional incidence of agranulocytosis in subjects receiving dapsone (DDS) prophylactically overseas and the contents of DDS and sulfone contaminants in the DDS tablets used, we compared the levels of these compounds in samples of various lots of DDS returned from overseas with the levels in other samples of the same lots that had been stored in U.S. depots.

As described in detail in Annual Report No. 1,[1] we used spectrophotometric, thin-layer chromatographic (TLC) and high-pressure liquid chromatographic (HPLC) procedures to analyze DDS tablets and DDS reference standards for DDS and sulfone contaminants. USP standard methods were also used for analysis of selected lots of DDS tablets. Eight contaminants were found in DDS tablets, five of which were identified; these were 4-amino-4'-chlorodiphenyl sulfone (I), 4-aminodiphenyl sulfone (II), 2,4'-diaminodiphenyl sulfone (III), 4-amino-4'-methylaminodiphenyl sulfone (IV), and 4-amino-4'-hydroxydiphenyl sulfone (VI). Two peaks found during HPLC and one contaminant found by TLC were not identified.

Four lots of DDS tablets selected for potential release for clinical use were found to conform to USP specifications. They were also found to contain the sulfone contaminants listed above. However, the sum content of Compounds I, II, III, and VI was only 1.10 to 1.89% of the total sulfones present. Compound IV was present in barely detectable amounts.

No striking or consistent qualitative or quantitative differences were noted in the contents of DDS or of any sulfone contaminant in samples of 12 lots of DDS tablets returned from overseas compared with the levels of sulfones found in other samples of the same lots that had been stored in U.S. depots. Therefore, these studies did not reveal the existence of sulfone contaminants unique to DDS tablets returned from overseas and suggested that the occurrence of agranulocytosis was not causally related to any of the components of the DDS tablets we have measured.

During the second contract year, we continued work on the identification of the three compounds of unknown structure found previously in DDS tablets. The major effort, however, was devoted to determining the effects of ultraviolet irradiation on pure DDS and on extracts of DDS tablets.

MATERIALS AND METHODS

Reference Compounds and Pharmaceutical Preparations of DDS

The DDS standard used in this work was the recrystallized product reported previously.[1] Sulfone derivatives used during this year but not listed in the last report[1] were:

- Compound V, 3,4'-diaminodiphenyl sulfone
- Compound IX, 4-amino-4'-nitrodiphenyl sulfone.

These compounds were provided by Dr. William T. Colwell of the Pharmaceutical Chemistry Department at SRI. Their preparation and characteristics were reported previously.[2]

A small amount of the yellow compound 3,6-diaminodibenzothiophene-9,9-dioxide (DDTD) was provided by Dr. Robert S. Rozman (WRAIR) as a possible product of the irradiation of DDS. The abstraction of hydrogen atoms from the 2 and 2' positions of DDS followed by ring closure could yield DDTD containing the five-membered thiophene dioxide ring. DDTD could be separated from DDS and other known sulfone contaminants of DDS tablets by TLC on glass plates precoated with Silica gel-60 (thickness 0.25 mm; E. M. Laboratories) using benzene:ethyl acetate (60:40) as the developing solvent. When the plates were exposed to ultraviolet light at 254 nm, DDTD exhibited yellow fluorescence at R_f 0.34. In solution, the following fluorescence characteristics were observed: maximum activation/emission wavelengths in methanol, 325/520 nm; in ethyl acetate, 325/470 nm. DDTD exhibited 22% and 38% of the fluorescence intensity of DDS at its maxima in methanol and ethyl acetate, respectively. It was not resolved from DDS by HPLC on Zipax-PAM.[1] Using a high-load capacity microparticulate silica gel column,* we found that DDTD exhibited a retention time of 10 min, which was earlier than the retention time of DDS at 21 min.

In current studies, we have been using mainly DDS tablets of Lots 111 CC, 438 CD, and 590 CD (Winthrop Laboratories) that had been stored at a depot in Tracy, California. Irradiation studies of tablets of Lot 438 CD that had been returned from overseas were also performed.

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*LiChrosorb SI-60, 5 μm (E. Merck), slurry-packed in a stainless-steel column measuring 4.6 × 250 mm. Developing solvent was benzene:ethyl acetate, 7:3, v/v, at a flow rate of 1.4 ml/min.

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Qualitative and Quantitative Analyses of Extracts of DDS Preparations

We used the procedures described previously[1] for the preparation of extracts of tablets, and for TLC and HPLC. Modifications of these techniques for specific purposes are described in the Results section.

Studies on the Photodecomposition of DDS Exposed to Ultraviolet Light

We conducted initial studies on the photodecomposition of DDS by UV light using an Aminco-Bowman spectrophotofluorometer equipped with a Xenon lamp. Quartz tubes (12 × 100 mm) containing DDS in ethylene dichloride were irradiated at 284 nm, and fluorescence was measured at 325 nm. The rate of decay was determined from the fluorescence observed at zero time and after subsequent 10-min intervals of irradiation. We also used HPLC to measure the actual loss of DDS from irradiated solutions. After discovering that loss of fluorescence of DDS was not a valid measure of the loss of DDS from solutions originally containing more than 10 μg DDS/ml, we abandoned this method. At that time, we substituted the simpler Turner fluorometer (Model No. 110) for the spectrophotofluorometer as the device for irradiating the samples. By use of the Turner fluorometer, DDS samples were irradiated at room temperature in quartz tubes (12 × 75 mm) situated 3.5 cm from an unfiltered 4-watt mercury lamp (General Electric No. G4T4/1).* The area of incidence light on the tube was 135 mm^2, and the light path to the phototube was blocked. Times of irradiation were as noted in the Results section, and all assessments of the loss of DDS were made by HPLC.

We determined the concentration of DDS in irradiated samples after evaporating a small aliquot to dryness under a stream of nitrogen in a 48° water bath. The residue was dissolved in ethyl acetate, and a small aliquot of this solution was injected onto a 3.2 × 250 mm column of microparticulate silica gel SI-60 using ethyl acetate at a flow rate of 0.45 ml/min for elution. All quantitative measurements were made by comparing peak heights of DDS from experimental samples with a calibration curve obtained by treating analytical DDS identically but omitting the irradiation step.

Rates of decay or half-times of disappearance ($T_{\frac{1}{2}}$) of DDS were calculated from regression equations of the logarithmic decrease of DDS with time. Comparisons between $T_{\frac{1}{2}}$ values obtained under various experimental conditions were made by assessing the significance of differences between slopes of the regression equations.[3]

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*We assessed the stability of emission of the lamp by reading daily the fluorescence of the same solution of quinine sulfate in 0.1 N sulfuric acid using primary filter 7-60, secondary filter 2A, and range setting at 1X. The coefficient of variation of numerous daily readings was 0.57%.

Gas Chromatography (GC) and Mass Spectrometry (MS)

Determinations of the structure of isolated compounds by GC-MS or by MS were performed under the direction of Dr. David Thomas, Senior Mass Spectroscopist, at SRI, using an LKB 9000 GC-MS equipped with a direct introduction probe and a DEC model PDP-12 computer. In most cases, samples were introduced directly for MS. In other cases, volatile trimethylsilyl derivatives were prepared and resolved by GC, with subsequent MS analysis. The sulfone contaminants were identified by comparison of their fragmentation patterns with those obtained from authentic compounds.

RESULTS

Studies on Contaminants in DDS Preparations

As reported previously,[1] three contaminants routinely seen in DDS preparations by TLC or HPLC were not identified. HPLC observations were that Peak V eluted between Compound IV (4-amino-4'-methylaminodiphenyl sulfone) and Compound VI (4-amino-4'-hydroxydiphenyl sulfone) and that Peak VII eluted after DDS. The third unknown exhibited a low R_f on TLC and blue fluorescence under UV light (365 nm), but no retention time was established during HPLC.

Additional work during this year resulted in the identification of Peak V, the partial elucidation of the structure of Peak VII, and the establishment of a retention time for the blue fluorescent compound, subsequently referred to as Peak VIII, after HPLC.

Peak V was identified through use of a combination of TLC, HPLC, and MS. Aliquots of the mother liquor from crystallization of DDS from $CHCl_3$ were subjected to TLC.[1] The front one-third of the DDS band was scraped off several plates, and the silica gel was extracted with ethyl alcohol. After evaporation of the solvent, the residue was taken up in $CHCl_3$. The mass fragmentation pattern of the $CHCl_3$ extract after GC showed that the major compounds present were DDS and its isomer 3,4'-diaminodiphenyl sulfone (Compound V), as confirmed by comparisons with the mass fragmentation patterns of the authentic compounds. In addition, we subsequently found that authentic Compound V exhibited the same retention time as Peak V on HPLC. We had previously reported semiquantitative estimates of the amounts of Peak V in various DDS preparations.[1] This contaminant accounted for only 0.03 to 0.08% of the total sulfones of DDS tablets, and the amounts were not different in DDS tablets of the same lot stored in U.S. depots or returned from overseas.

The material that eluted as Peak VII was not resolved from DDS by TLC. However, its retention time was longer than that of DDS during HPLC.[1] Numerous aliquots from the same mother liquor used for the crystallization of Compound V were subjected to HPLC using 20% ethyl alcohol and 0.4% water in hexane as the eluting solvent at a flow rate of 1.0 ml/min. Under these conditions, DDS exhibited a retention time of 5.9 min, whereas Peak VII was retained 14.8 min. Numerous fractions containing Peak VII were collected, pooled, and evaporated to dryness. Column chromatography of the residue indicated that the major components present were DDS and Peak VII in approximately equal amounts. During GC, Peak VII was found to be much less volatile than DDS. Its mass fragmentation pattern was complex and showed a parent ion of molecular weight 480. A compound of this molecular weight could be formed by dimerization of DDS, but we could not assign

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a structure to it with certainty. We previously reported[1] that Peak VII was present in DDS tablets to the extent of 0.05 to 0.09% of the total sulfones. As with all other contaminants of DDS tablets, we found no differences in the amounts of Peak VII in samples of the same lots of DDS that had been returned from overseas or had been stored in U.S. depots.

The blue fluorescent material (Peak VIII) previously had been concentrated by "dry-column" chromatography.[1] Aliquots of this sample enriched in Peak VIII were subjected to HPLC under the conditions described above for the separation of DDS and Peak VII. Fractions were collected every 5 min and evaporated to dryness, and the residue was subjected to TLC until Peak VIII was located (retention time, 53 min). Numerous fractions from several chromatographic separations were pooled and evaporated to dryness. The UV spectrum in ethyl alcohol showed a major absorption peak at 276 nm and a second, less intense, peak at 342 nm. These characteristics are substantially different from those of the available sulfones, which exhibit maxima at 300 nm or less in ethanol and no peaks in the region greater than 300 nm. On direct-probe analysis for MS, no volatile compound could be detected. These results suggested that Peak VIII was very different from all the sulfones previously identified because they all yield volatile compounds on direct-probe analysis by MS. In an earlier GC-MS analysis of a crude preparation of Peak VIII material,[1] no sulfone derivative was detected either. We performed no further work to establish the identity of this contaminant.

Studies of the Effects of Ultraviolet Irradiation on DDS in Organic Solvents

Previously, we had examined the stability of DDS in ethylene dichloride during UV irradiation to establish the time available to read samples for the routine analysis of DDS by fluorometry. From that assessment, we found that DDS lost fluorescence rapidly and calculated a $T\frac{1}{2}$ of less than 1 hr for DDS.

Our initial effort in this contract work was to study the relationship between the concentration of DDS in ethylene dichloride and the $T\frac{1}{2}$ of the fluorescence of DDS. The DDS used was the recrystallized preparation described previously.[1] Concentrations of DDS ranging from 0.1 to 5000 μg/ml were examined by continuous monitoring of the fluorescence during exposure in the Aminco-Bowman spectrophotofluorometer for 60 to 150 min. The $T\frac{1}{2}$ values were calculated from the fluorescence observed at zero time and at subsequent 10-min intervals. Table 1 presents the results obtained. The $T\frac{1}{2}$ at 0.1 μg/ml was 0.97 hr, and it increased to 4.9 hr at 10 μg DDS/ml. However, at increasing DDS concentrations, the $T\frac{1}{2}$ value decreased gradually to 0.87 hr at 5000 μg DDS/ml.

In an attempt to understand these unusual results, we determined the actual remaining DDS in the irradiated solutions by HPLC when the fluorescence had decreased to 50% of its zero-time value. Table 2 shows the results obtained using four concentrations of DDS. In samples containing

Table 1

HALF-TIME OF DISAPPEARANCE OF THE FLUORESCENCE OF DDS IN ETHYLENE DICHLORIDE DURING ULTRAVIOLET IRRADIATION

DDS Concentration (μg/ml)[a]	Half-time of Disappearance (hr)[b]
0.1	0.97
1	1.8
10	4.9
100	2.4
1000	1.3
5000	0.87

[a]Samples in quartz tubes were irradiated in an Aminco-Bowman spectrophotofluorometer at 284 nm. Fluorescence was measured at 325 nm.

[b]Calculated from the regression lines of the logarithmic decay of the fluorescence of DDS with time. All regression lines exhibited r values ≤ -0.986.

Table 2

COMPARISON OF THE LOSS OF DDS DURING ULTRAVIOLET IRRADIATION BY DECREASED FLUORESCENCE AND MEASURED DDS CONTENT

DDS Concentration (μg/ml)[a]	Relative Fluorescence (%)[b]	DDS Content (%)[c]
1	52	43
10	49	37
1000	50	95
5000	50	98

[a]Same as footnote a, Table 1.

[b]Percentage of the fluorescence of the DDS solution at zero time.

[c]Percentage of the DDS content of the solution at zero time determined by microbore column chromatography.

1 and 10 μg DDS/ml, we found that the actual loss of DDS essentially agreed with the loss of fluorescence. However, the samples containing 1000 and 5000 μg DDS/ml exhibited practically no loss of DDS content, yet the fluorescence measurements indicated a loss of 50%. We believe these results indicate quenching of the fluorescence of DDS by minute amounts of photodecomposition products at high DDS concentrations rather than extensive photodecomposition of the DDS.

These results suggested that the measured fluorescence of a DDS solution more concentrated than 10 μg/ml may not be a valid index of the DDS concentration in samples subjected to UV irradiation. Therefore, we subsequently used HPLC to measure DDS content so as to assess the loss of DDS in several solvents. For irradiation in these and most of the subsequent studies, we used the much simpler and less expensive Turner filter fluorometer.

We determined loss of DDS in ethylene dichloride, ethyl acetate, and ethyl alcohol by taking small aliquots of the irradiated solutions of DDS at various times. These were evaporated to dryness under nitrogen, and the remaining DDS was measured by HPLC. Table 3 presents the $T_{\frac{1}{2}}$ values found for two concentrations of DDS in the three solvents. At approximately 100 μg/ml, we obtained a $T_{\frac{1}{2}}$ value of 1.6 hr in ethylene dichloride but values of 6.4 and 4.5 hr in ethyl acetate and ethyl alcohol, respectively. A similar effect of the solvent was noted at approximately 1000 μg DDS/ml, with a much shorter $T_{\frac{1}{2}}$ in ethylene dichloride than in ethyl acetate and ethyl alcohol. But at this concentration, loss of DDS was markedly slower than at 100 μg/ml.

During HPLC of these irradiated solutions for the measurement of loss of DDS, we did not observe concomitant increases of any of the DDS contaminants identified previously. However, TLC of the irradiated solutions showed the presence of UV-absorbing materials that exhibited lower R_f values than any previously observed. From the conditions used for TLC, these unknown materials would be expected to be more polar derivatives of DDS than any of the known contaminants.

To investigate the difference in the rate of loss of DDS in the three solvents, we irradiated the solvents for various periods before mixing them with a solution containing DDS in the same type of nonirradiated solvent, as described in Footnote b of Table 4. We used the fluorescence of the resulting mixtures to assess loss of DDS. Use of ethylene dichloride that had been irradiated for only 0.5 hr gave only 7% of the fluorescence expected from the DDS content. Ethylene dichloride that had been irradiated longer produced an even greater loss of the theoretical fluorescence (Table 4). This phenomenon was not as pronounced in ethyl acetate, wherein preirradiation for 16 hr was required to cause a loss of 47% of the theoretical fluorescence of the DDS. Irradiation of ethyl alcohol for up to 16 hr had no effect on the fluorescence of DDS.

To test the validity of the very rapid loss of the fluorescence of DDS in ethylene dichloride, we examined by HPLC the DDS content of the sample prepared from ethylene dichloride that had been preirradiated for

Table 3

HALF-TIME OF DISAPPEARANCE OF DDS IN VARIOUS SOLVENTS DURING ULTRAVIOLET IRRADIATION

Solvent[a]	DDS Concentration (μg/ml)	Half-time of Disappearance (hr)[b]
Ethylene dichloride	89.4	1.6
	894	38
Ethyl acetate	103	6.4
	1030	81
Ethyl alcohol	102	4.5
	1020	108

[a]DDS in the solvents was irradiated in a quartz tube with a 4-watt mercury lamp (GE No. G4T4/1) in a Model No. 110 Turner fluorometer. No primary filter was used, and the incident light slit was opened maximally.

[b]DDS content at various time periods was measured by microbore column chromatography. Half-times were calculated from regression lines of the logarithmic disappearance with time.

Table 4

EFFECT OF IRRADIATING SOLVENTS ON THE FLUORESCENCE OF DDS

Irradiation Time (hr)[a]	Relative DDS Fluorescence in[b]		
	Ethylene Dichloride	Ethyl Acetate	Ethyl Alcohol
0	100	100	100
0.5	7	—	—
1	4	—	—
2	2	95	100
4	1	85	100
8	—	69	—
16	—	53	100

[a]Pure solvent was irradiated under the conditions of footnote a in Table 3.

[b]Solutions of 100 μg DDS/ml in each solvent were diluted 1:10 with each respective irradiated solvent. The fluorescence of the resulting solution was measured in the Aminco-Bowman spectrophotofluorometer using activation/emission wavelengths of 284/325 nm.

2 hr. Only 6% of the theoretical DDS was found, confirming the loss assessed by fluorescence.

The known ability of UV radiation to induce formation of free radicals in halogenated hydrocarbons leads us to believe that the short $T\frac{1}{2}$ values observed for DDS in ethylene dichloride are due to the summation of two processes: (a) the direct effect of irradiation on DDS and (b) the effect of the free radicals induced in the solvent on DDS. This study clearly indicates that, to measure only the effect of irradiation on DDS, we must use, of the three solvents examined, ethyl alcohol.

Next, we examined the possible influence of DDS contaminants and ethanol-soluble tablet excipients on the rate of disappearance of DDS during irradiation. To accomplish this, we compared the rate of loss of pure DDS from an ethanolic solution containing added sulfone compounds (in amounts similar to the levels of sulfone contaminants found in tablets) with the rate of loss of DDS from ethanolic extracts of two lots of DDS tablets. The DDS content of the solutions was determined by HPLC on Zipax-PAM after hourly intervals of irradiation using the Turner fluorometer.

Table 5 lists the percentages of the original solutions, each of which contained 100 μg DDS/ml, found after irradiation. Only mean values are presented because the variability of individual values was always less than 2% of the mean. The half-lives of 5.3 and 5.4 hr (see last column of table) for two tests using the mutual standard were similar to the value of 4.5 hr reported previously for pure DDS without added sulfone contaminants. Also, the ethanolic extract of tablets of Lot 438 CD that had been stored in the United States or returned from overseas gave nearly the same half-lives as those found for the mutual standard. These results do not suggest any differences in susceptibility to irradiation between pure DDS mixed with very small amounts of sulfone derivatives and DDS derived from tablets stored overseas or in the United States. The last entry of Table 5 shows that Lot 590 CD exhibited longer half-lives than the other DDS samples tested in this group. The DDS in this lot evidently was more resistant to the effects of irradiation than was Lot 438 CD or the mutual standard.

To examine the possible role of dissolved oxygen in the irradiated solvent containing DDS, we performed three tests on an ethanolic extract of a tablet of Lot 590 CD. These are described and summarized in Table 6. For brevity, mean values of the triplicate observations are presented. However, for calculation of regression lines and $T\frac{1}{2}$ values, all values were used. None of the slopes of the three regression lines were significantly different from each other, indicating that saturation of the ethanolic extract of this DDS tablet with oxygen or nitrogen had no influence on the decay rate of DDS due to irradiation.

Examination by HPLC[1] of aliquots of irradiated ethanolic solutions of pure DDS (100 μg/ml) revealed the presence of several new peaks in addition to the contaminants found previously. A major peak with a retention time of 15 min occurred shortly after 4-aminodiphenyl sulfone was

Table 5

RATES OF DISAPPEARANCE OF DDS AFTER IRRADIATION WITH ULTRAVIOLET LIGHT

Sample[a]	Percentage of DDS Remaining after Irradiation[b]								Half-life (hr)
	1 hr	2 hr	3 hr	4 hr	5 hr	6 hr	7 hr	8 hr	
Mutual Standard	99.0	85.8[c]	77.9	71.5	55.5[c]	47.5	—[d]	—[d]	5.3
(Repeat)	—[d]	97.3	85.6	75.3	62.0	55.5	45.2	29.8[c]	5.4
Lot 438 CD	87.6	75.7	63.5	54.7	48.6	42.8	36.4	—[d]	4.8
(Repeat)	87.7[c]	80.8	75.6	59.9	61.1	47.1	42.7	36.1	5.5
Lot 438 CD[e]	91.9	73.6	65.8	60.7	51.7	40.5[c]	34.5	30.0	4.6
(Repeat)	94.9	93.6[c]	84.3	74.9	70.7	65.7	54.3	49.9	7.8
Lot 590 CD	95.1	94.2	82.1	78.2	—[d]	71.1	70.2	—[d]	12.6
(Repeat)	99.1	94.9	93.8	86.2	80.2	79.1	70.3	—[d]	13.8

[a]All tablets were from Tracy, CA, unless otherwise noted. The mutual standard consisted of pure DDS spiked with the four sulfone contaminants previously identified in amounts similar to levels found in tablets.[1]

[b]Two ml of ethanol containing 100 μg DDS/ml were irradiated in the Turner fluorometer. At the indicated time intervals, 10-μl aliquots were removed and the DDS content determined by HPLC. Values are means of triplicate determinations unless otherwise noted.

[c]Mean of duplicate determinations.

[d]Sample not taken.

[e]Sample from Vietnam.

Table 6

INFLUENCE OF NITROGEN OR OXYGEN ON DDS DISAPPEARANCE AFTER ULTRAVIOLET IRRADIATION

Treatment of Ethanol[a]	Mean Percentage of DDS Remaining After Irradiation								Half-life (hr)
	0 hr	1 hr	2 hr	3 hr	4 hr	5 hr	6 hr	7 hr	
None	100	95.1	94.2	82.1	78.2	—[b]	71.1	70.2	12.6
Saturation with N_2	100	99.7	81.4	78.5	78.0	79.3	76.0	72.0	15.0
Saturation with O_2	100	91.9	80.4	76.7	—[b]	73.6	75.6	74.5	18.9

[a]Ethanolic extracts of a DDS tablet (Lot No. 590 CD, Tracy, CA) were irradiated in the Turner fluorometer. Prior to irradiation, one aliquot of the tablet extract was not treated, and two others were evaporated to dryness; the residues obtained were dissolved in ethanol that had been gassed for 30 min with N_2 or O_2. The concentration of DDS was 100 μg/ml, and triplicate aliquots were taken after the various periods of irradiation and assayed by HPLC.

[b]Sample not taken.

eluted. In addition, three new minor peaks were observed in all samples. The first eluted immediately before and the second immediately after 2,4'-diaminodiphenyl sulfone. The third small peak was eluted after DDS. This same general pattern of new peaks was found when HPLC was applied to aliquots of ethanolic solutions containing 1000 μg of DDS/ml after irradiation.

To isolate sufficient quantities of the new major peak (IX) for analysis, we used for HPLC a column of microparticulate silica gel instead of the usual polyamide (Zipax-PAM) because silica gel has a greater load capacity. In addition, use of a silica gel column allowed much more rapid analyses because it yielded shorter retention times; for example, DDS was retained 21 min on the silica column and 60 min on the polyamide column. However, the order of elution of sulfones and unknowns was the same on both columns.

After irradiating 2 ml of an ethanolic extract of a DDS tablet (1000 μg/ml, Lot 111 CC), we evaporated the ethanol and dissolved the residue in running solvent; then the residue was subjected to HPLC on microparticulate silica gel. The large unknown Peak IX (retention time, 6 min) eluting shortly after 4-aminodiphenyl sulfone was collected; after evaporation of the solvent, the residue was subjected to GC-MS. Direct probe analysis yielded a fragmentation pattern identical to that of authentic 4-amino-4'-nitrodiphenyl sulfone (Compound IX). When this reference compound was subjected to HPLC on a polyamide column, it had the same retention time (15 min) as the major peak from the irradiated ethanolic DDS solutions.

The new minor peaks found after irradiation of DDS in ethanol were not identified. However, the compound, DDTD, provided by Dr. Rozman, exhibited a retention time after HPLC on microparticulate silica gel similar to that of one of these unknown minor peaks.

To measure the relationship between loss of DDS and formation of 4-amino-4'-nitrodiphenyl sulfone, we irradiated ethanolic solutions of pure DDS mixed with the sulfone contaminants of tablets and ethanolic extracts of DDS tablets. After irradiating solutions containing 100 μg of DDS/ml and 1000 μg of DDS/ml for 6 and 17 hr, respectively, we measured the loss of DDS by HPLC on Zipax-PAM. The amount of the nitro derivative produced was determined using calibration runs of authentic 4-amino-4'-nitrodiphenyl sulfone; that is, peak heights were used to assess levels of the compound in experimental samples.

As indicated in Table 7, using the mutual standard we found a much greater percentage of loss of DDS at 100 μg/ml than at 1000 μg/ml. At both DDS concentrations, the measured amount of the nitro derivative produced accounted for only approximately one-fourth of the DDS lost. Similar results were obtained after irradiation of the ethanolic extracts of tablets--that is, a greater percentage of DDS was lost at the lower starting concentration, and the amount of nitro derivative produced never accounted for more than 50% of the loss of DDS.

The changes in levels of DDS contaminants in these samples were also measured, and Table 8 presents these values. No values for 4-amino-4'-hydroxydiphenyl sulfone are presented because this compound was not resolved from DDS using this HPLC system. The only statistically significant change caused by irradiation of the samples containing 100 μg of DDS/ml was a mean loss of 0.24 μg of 2,4'-diaminodiphenyl sulfone/ml. At a concentration of 1000 μg of DDS/ml, no mean losses were found. A mean increase of 1.7 μg of 4-aminodiphenyl sulfone/ml was observed, however. This suggests that some of this compound had been produced from DDS during irradiation.

Studies of the Effects of Ultraviolet Irradiation on DDS in Water

Initial studies on the photodecomposition of DDS in water were performed on an aliquot of an ethanolic extract of a DDS tablet (Lot No. 111 CC); the extract was evaporated to dryness, and the residue was dissolved in water for irradiation. Panel C of Figure 1 shows the elution profile from this sample after it was subjected to HPLC on LiChrosorb SI-60. Peaks XII and XIII, which were not observed previously, are identified below. For comparison, Panel B shows the elution profile of an aliquot of the ethanolic extract of this tablet of DDS after irradiation. In this run, Peaks I (4-amino-4'-chlorodiphenyl sulfone) and IX (4-amino-4'-nitrodiphenyl sulfone) were not resolved. Peaks X and XI and the three minor peaks eluting after Peak III (2,4'-diaminodiphenyl sulfone) have not been identified. No further work on establishing the identity of or on quantitating products of the photodecomposition of DDS in ethanol was done because of the higher priority of studies on DDS photodecomposition in water.

To prepare sufficient quantities of Peaks XII and XIII for structural investigation, we collected the fractions corresponding to these peaks from several chromatographic separations involving the total injection of approximately 1.0 mg of DDS. Samples for HPLC were aliquots of an irradiated aqueous solution containing 100 μg of DDS/ml derived from an ethanolic extract of a DDS tablet of Lot 111 CC. Irradiation was for 1 hr in the Turner fluorometer. The respective fractions of Peak XII and XIII were pooled and evaporated to dryness under nitrogen in a 48° water bath.

The residues were dissolved in 2 ml of methanol to allow determination of UV absorption and fluorescence characteristics. Subsequently, we evaporated these solutions to dryness under nitrogen and redissolved the residues in 2 ml of ethyl acetate to redetermine the fluorescence characteristics. Table 9 lists these values as well as those for authentic DDS. The UV absorption and fluorescence characteristics of Peaks XII and XIII were very similar to those of DDS, suggesting that the peaks were diaryl sulfones. However, the relative fluorescence of Peaks XII and XIII in the two solvents differed markedly from DDS and from each other.

After evaporation of the ethyl acetate, the residues from the pooled fractions of Peaks XII and XIII were subjected to analysis by MS. Figure 2 summarizes the fragmentation pattern obtained from Peak XIII. From the major ions found, we concluded that Peak XIII was 4-amino-4'-(2-phenylhydrazino)diphenyl sulfone because the pattern obtained was characteristic

Table 7

EFFECT OF IRRADIATION ON DDS AND TABLET EXTRACTS IN ETHANOL AND THE PRODUCTION OF 4-AMINO-4'-NITRODIPHENYL SULFONE

Sample No.	Source[a]	Concentration Irradiated (μg/ml)[b]	Loss of DDS (%)	Amount of 4-Amino-4'-nitrodiphenyl Sulfone Formed (μg/ml irradiated solution)
I	Mutual	100	34.6	7.2
	Standard	1000	3.5	12
II	Lot No. 111 CC	100[c]	35.7	5.7
		1000[c]	—[d]	14
		1000	10.2	13
		1000	13.2	12
III	Lot No. 438 CD	100[c]	40.5	10
		100[e]	20.6	4.8
		1000[c]	2.6	13
IV	Lot No. 590 CD	1000	5.3	15

[a]Tablets of DDS were from Tracy, CA, unless otherwise noted. The mutual standard was prepared as described in Table 5.

[b]Solutions of 100 and 1000 μg DDS/ml of ethanol were irradiated in the Turner fluorometer for 5.8 and 17 hr, respectively.

[c]Aliquots of the same tablet extract. All others were extracts of different tablets.

[d]Not determined.

[e]Vietnam sample.

Table 8

EFFECT OF IRRADIATION ON THE CONCENTRATION OF SULFONE CONTAMINANTS

Sample No.[a]	Concentration Irradiated (μg/ml)[a]	Contaminant Concentration (μg/ml)[b]								
		4-Amino-4'-chlorodiphenyl Sulfone			4-Aminodiphenyl Sulfone			2,4'-Diaminodiphenyl Sulfone		
		Pre	Post	Change	Pre	Post	Change	Pre	Post	Change
I	100	0.73	0.80	0.07	0.87	1.0	0.13	0.83	0.53	-0.30
II	100[c]	0.29	0.24	-0.05	0.89	0.63	-0.26	0.97	0.61	-0.36
III	100[c]	0.14	0.30	0.16	0.78	1.1	0.32	0.90	0.70	-0.20
	100[d]	0.17	0.35	0.18	0.92	1.2	0.28	0.91	0.83	-0.08
		Mean Change		0.09			0.12			-0.24[e]
I	1000	2.6	3.3	0.7	3.7	5.7	2.0	3.7	2.9	-0.8
II	1000[c]	3.2	3.9	0.7	8.6	12	3.4	9.5	7.2	-2.3
	1000	3.2	3.0	-0.2	10	9.6	-0.4	7.6	9.1	1.5
	1000	2.6	2.6	0.0	11	12	1.0	9.2	11	1.8
III	1000[c]	1.4	2.1	0.7	6.4	9.6	3.2	9.8	9.8	0.0
IV	1000	2.2	2.4	0.2	8.7	9.6	0.9	9.1	10	0.9
		Mean Change		0.4			1.7[e]			0.2

[a] Samples are identified in Table 7.

[b] Values are μg contaminant found/ml of ethanol in pre- and post-irradiated samples.

[c] Aliquots of the same tablet extract; all others were extracts of different tablets.

[d] Vietnam sample.

[e] Irradiated samples were significantly ($P < 0.05$) different from nonirradiated samples. All other mean changes were not significantly different.

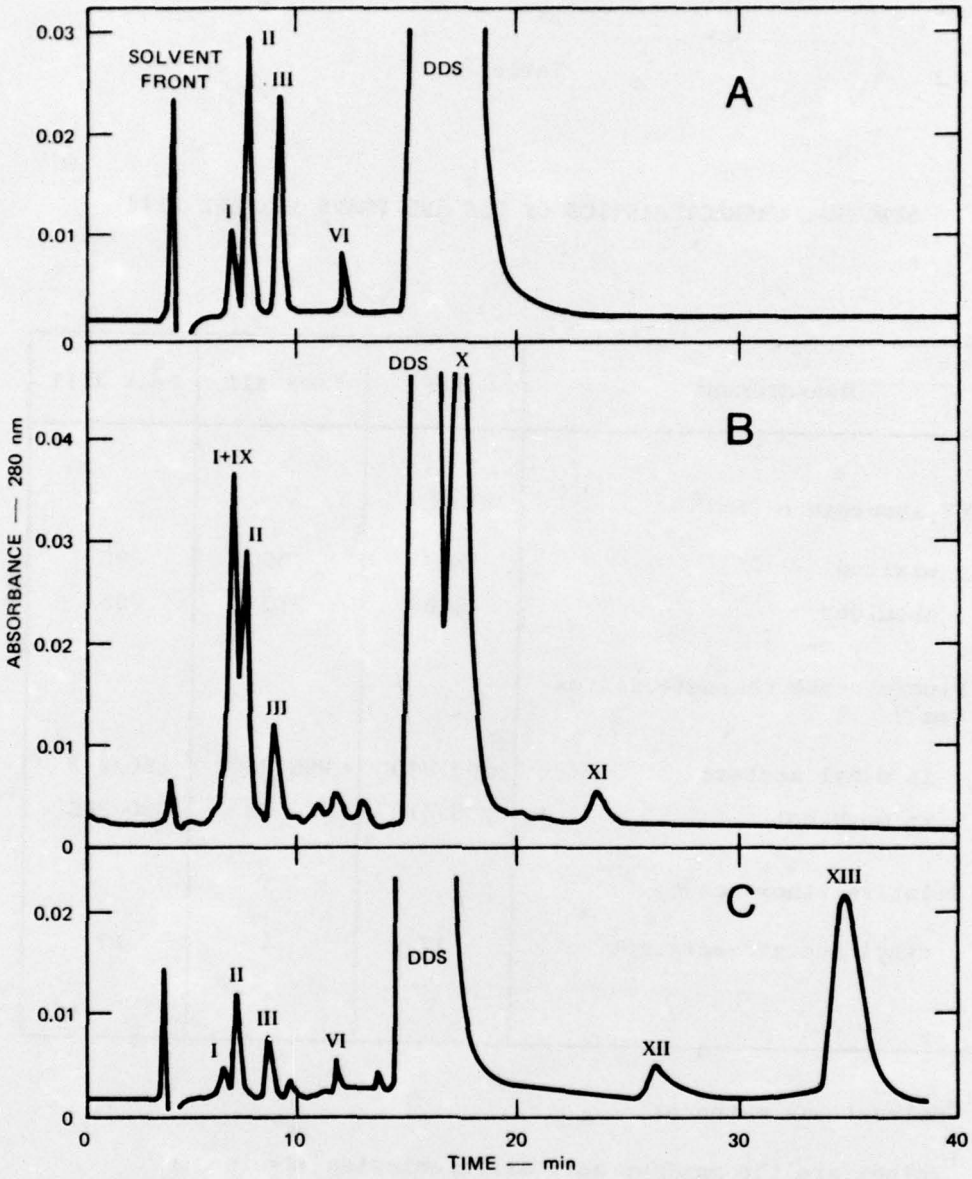

FIGURE 1 ELUTION PROFILES OBTAINED FROM HPLC ON LiCHROSORB Si-60 OF AN ETHANOLIC EXTRACT OF A DDS TABLET (LOT NO. 111 CC)

(A) Before irradiation, 12 μg of DDS chromatographed; (B) After irradiating 100 μg of DDS/ml of ethanol for 6 hr; (C) After irradiating 100 μg DDS/ml of water for 1 hr. Peaks identified are I, 4-amino-4'-chlorodiphenyl sulfone; II, 4-aminodiphenyl sulfone; III, 2, 4'-diaminodiphenyl sulfone; VI, 4-amino-4'-hydroxydiphenyl sulfone; and IX, 4-amino-4'-nitrodiphenyl sulfone.

Table 9

SPECTRAL CHARACTERISTICS OF DDS AND PEAKS XII AND XIII

Measurement	DDS	Peak XII	Peak XIII
UV absorbance (nm)[a]			
maximum	296	305	295
shoulder	259	270	255
Fluorescence characteristics (nm)[b]			
in ethyl acetate	295/340	290/335	290/375
in methanol	300/410	295/385	300/385
Relative fluorescence			
ethyl acetate/methanol	17	2	27

[a]Solvent was methanol.

[b]Values are the maximum activation/emission wavelengths.

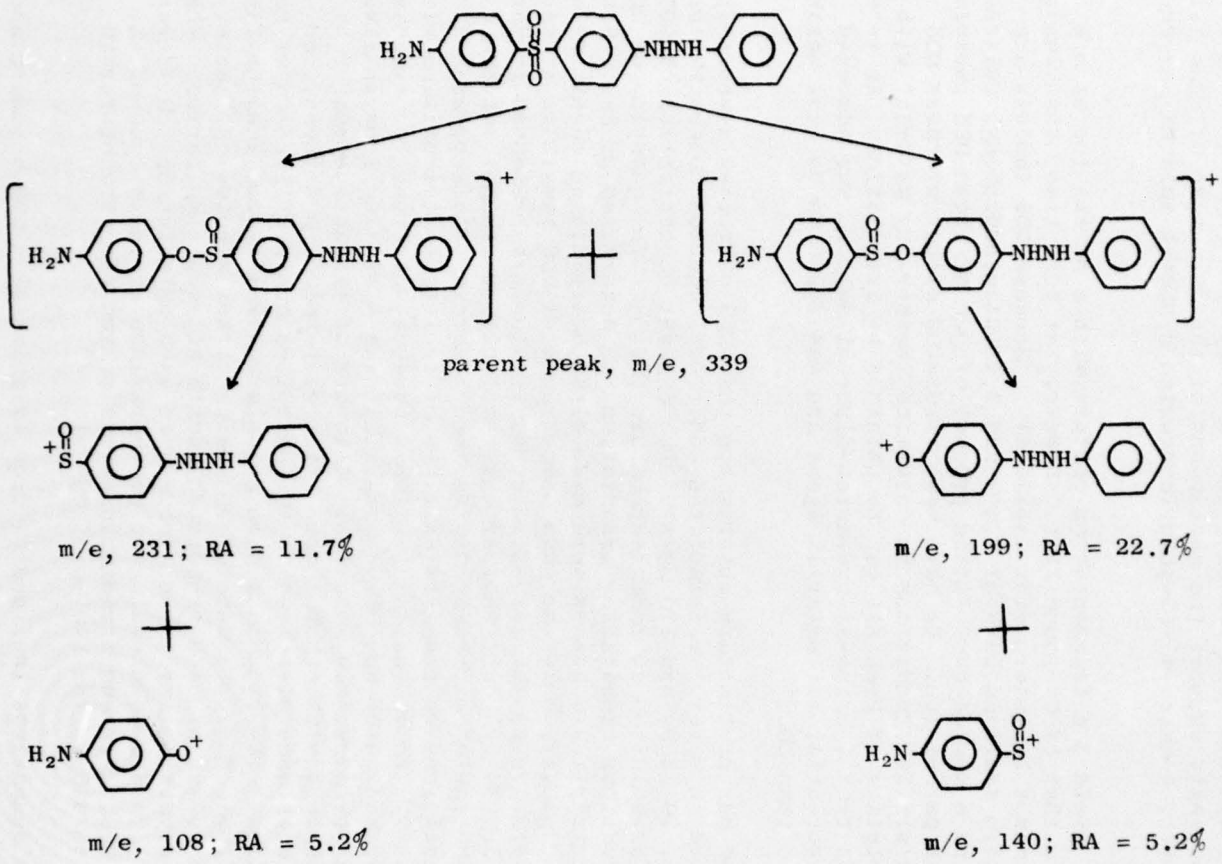

Figure 2 MASS FRAGMENTATION PATTERN OF COMPOUND XIII
Relative Abundance (RA) is the percentage
of the Parent Peak

of those obtained from unsymmetrical diaryl sulfones.[4] In this fragmentation process, the parent sulfone ion rearranges to form isomeric aryl arenesulfinate esters, which are detected as the parent ion (m/e, 339). Rupture of the S-O bond then occurs, producing two different arylsulfoxy ions (m/e, 231 and 140) and two different aryloxy ions (m/e, 199 and 108). These facts strongly support the assignment of the structure of Peak XIII to Compound XIII, 4-amino-4'-(2-phenylhydrazino)diphenyl sulfone, as shown in Figure 2.

Peak XII yielded a fragmentation pattern with a parent ion at m/e 339 similar to that of Compound XIII. However, at this time, the remaining pattern cannot be interpreted explicitly. Because DDS tablets are known to contain small amounts of 2,4'- and 3,4'-diaminodiphenyl sulfones, Peak XII may be a photodecomposition product of one of these DDS isomers and yield this parent ion. We have not irradiated either of these DDS isomers under similar conditions to determine whether any material with the characteristics of Peak XII can be obtained by irradiation. An interesting note is that 4-amino-4'-hydrazino-diphenyl sulfone was screened earlier as a potential antimalarial agent and was found to be less active and more toxic than DDS.[5]

To extend our preliminary observation that DDS exhibited a short $T\frac{1}{2}$ when irradiated in water, we irradiated additional aqueous concentrations of pure DDS of 10, 100, and 170 μg/ml. The highest concentration of DDS was a saturated solution at room temperature (22°C). The quantity of DDS remaining at various times after irradiation was determined by HPLC. Single, duplicate, or triplicate assays were made of each irradiated sample at each time period. However, only the mean percentages of DDS remaining at each time period relative to the zero-time concentration are presented in Tables 10, 11, and 12. To evaluate whether water-soluble or ethanol-soluble material from the tablet excipients influenced the rate of disappearance of DDS upon irradiation, we prepared extracts of tablets by trituration with ethanol or water. In all cases, the final aqueous solutions for irradiation were prepared using deionized water that was distilled from an alkaline potassium permanganate solution. Aliquots of extracts prepared by triturating tablets with ethanol were first evaporated to dryness, and then the residues were dissolved in the deionized and distilled water before irradiation. The $T\frac{1}{2}$ values were calculated from the regression lines of the logarithmic disappearance of DDS using either single, duplicate, or triplicate assays of each irradiated sample at each time period. The zero-time observations were also included in each calculation. Differences between $T\frac{1}{2}$ values were evaluated by comparison of the slopes of the regression lines. Maximum irradiation times ranged from 1.0 hr for 10 μg of DDS/ml to 6.0 hr for 170 μg of DDS/ml.

Table 10 summarizes the results from irradiating a DDS standard and aqueous or ethanolic extracts of tablets from Lots 111 CC and 590 CD at concentrations of approximately 10 μg/ml. More than one entry for any given lot represents a different $T\frac{1}{2}$ determination for the same tablet extract. Columns 7 and 8 of Table 10 show the various $T\frac{1}{2}$ values and the correlation coefficients of the regression lines. The $T\frac{1}{2}$ of DDS from aqueous extracts of tablets ranged from 0.31 hr to 0.58 hr; from ethanolic

Table 10

DISAPPEARANCE OF DDS DURING ULTRAVIOLET IRRADIATION
10 μg/ml of Water

Sample or Lot	Hours of Irradiation[a]					$T_{\frac{1}{2}}$ (hr)[b]	Correlation Coefficient
	0	0.25	0.50	0.75	1.0		
DDS Standard	100	67.3	44.0	24.5	16.3	0.37	-0.9976
---------- Aqueous Extract of Tablet[c] ----------							
111 CC	100	60.0	35.3	17.1	11.6	0.31	-0.9966
111 CC	100	82.5	65.4	43.8	31.0	0.58	-0.9893
111 CC	100	70.2	46.8	30.0	18.7	0.41	-0.9981
590 CD	100	70.0	47.4	33.6	22.3	0.46	-0.9996
590 CD	100	69.1	48.7	34.0	21.6	0.46	-0.9987
---------- Ethanolic Extract of Tablet[c] ----------							
111 CC	100	60.0	45.2	29.6	17.6	0.41	-0.9958
111 CC	100	45.1	30.3	14.3	—[d]	0.28	-0.9927
590 CD	100	79.6	25.6	16.2	4.9	0.23	-0.9796
590 CD	100	41.1	17.5	13.9	3.8	0.23	-0.9834

[a] Values are percentages of DDS remaining. Assays of the DDS Standard and the water-extracted tablets were performed in duplicate and means are listed; all others were single determinations. Multiple entries for a lot represent different trials on the same tablet extract.

[b] Values were calculated from the regression lines of the logarithmic disappearance of DDS using all available data points.

[c] Extracts were prepared by triturating tablets. Aliquots of the ethanolic extracts were evaporated to dryness and the residue dissolved in water prior to irradiation. Final concentrations ranged from 9.5 to 10.3 μg DDS/ml of water.

[d] Not assayed.

Table 11

DISAPPEARANCE OF DDS DURING ULTRAVIOLET IRRADIATION
100 μg/ml of Water

Sample or Lot	Hours of Irradiation[a]									$T_{\frac{1}{2}}$ (hr)[b]	Correlation Coefficient
	0	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0		
DDS Standard	100	84.2	74.5	58.3	48.3	40.3	29.3	22.6	-[d]	1.6	-0.9924
DDS Standard	100	90.3	77.6	64.7	54.1	42.6	-[d]	25.1	19.5	1.7	-0.9895
Aqueous Extract of Tablet[c]											
111 CC	100	96.6	86.6	78.9	-[d]	52.8	44.1	39.3	32.2	2.3	-0.9870
590 CD	100	88.7	75.0	63.8	51.7	41.8	32.0	24.6	17.3	1.6	-0.9894
590 CD	100	94.2	85.9	76.9	63.7	54.6	46.8	40.1	-[d]	2.5	-0.9905
590 CD	100	87.9	82.0	71.4	61.2	53.3	44.2	35.4	26.6	2.3	-0.9889
Ethanolic Extract of Tablet[c]											
111 CC	100	84.2	72.9	60.6	-[d]	41.1	32.3	23.5	-[d]	1.7	-0.9936
590 CD	100	87.8	71.6	62.0	-[d]	43.7	35.4	28.8	22.1	1.9	-0.9945

[a]Same as footnote a of Table 10 except that all values are the means of triplicate assays.

[b]Same as footnote b of Table 10.

[c]Same as footnote c of Table 10 except that the concentrations irradiated ranged from 97.7 to 100 μg DDS/ml of water.

[d]Not assayed.

Table 12

DISAPPEARANCE OF DDS DURING ULTRAVIOLET IRRADIATION
170 μg/ml of Water

Sample or Lot	Hours of Irradiation[a]							$T_{\frac{1}{2}}$ (hr)[b]	Correlation Coefficient
	0	1.0	2.0	3.0	4.0	5.0	6.0		
DDS Standard	100	91.2	75.8	61.0	51.6	42.6	33.7	3.8	-0.9955
DDS Standard	100	87.0	72.1	-[d]	49.7	40.1	35.5	3.9	-0.9965
Aqueous Extract of Tablet[c]									
111 CC	100	84.4	79.4	-[d]	57.5	46.5	40.5	4.6	-0.9932
590 CD	100	82.8	69.8	59.4	48.6	39.9	-[d]	3.8	-0.9949
Ethanolic Extract of Tablet[c]									
111 CC	100	88.7	72.6	59.9	49.0	35.5	28.0	3.2	-0.9907
590 CD	100	85.2	73.6	58.1	48.0	36.4	27.5	3.2	-0.9919

[a]Same as footnote a, Table 11.

[b]Same as footnote b, Table 10.

[c]Same as footnote c of Table 10, except that the concentrations irradiated ranged from 161 to 173 μg DDS/ml of water.

[d]Not assayed.

extracts, they ranged from 0.23 to 0.41 hr. The DDS standard yielded a $T\frac{1}{2}$ of 0.37 hr, which was similar to the values obtained from either aqueour or ethanolic extracts.

At a concentration of approximately 100 μg/ml, the $T\frac{1}{2}$ increased to values that ranged from 1.6 hr for the DDS standard to 2.5 hr for the aqueous extract of a tablet from Lot 590 CD. These results, summarized in Table 11, show that the $T\frac{1}{2}$ of the DDS standard increased severalfold from 0.37 hr at 10 μg/ml to 1.6 or 1.7 hr at 100 μg/ml. There was little difference between the $T\frac{1}{2}$ of the DDS standard and the $T\frac{1}{2}$ of DDS obtained from either ethanolic or aqueous extracts of tablets. Increasing the concentration of DDS in water to near the saturation point of approximately 170 μg/ml lengthened the average $T\frac{1}{2}$ of DDS to 3.8 hr (Table 12). Again, no pronounced differences were noted between the $T\frac{1}{2}$ of the DDS standard and the $T\frac{1}{2}$ of DDS for either aqueous or ethanolic extracts of tablets.

For a more rigorous comparison of the $T\frac{1}{2}$ of DDS as affected by either concentration or method of tablet extraction, a single $T\frac{1}{2}$ was calculated for each starting concentration using all available data points for that particular sample. Table 13 summarizes the results. At a concentration of 10 μg/ml, Lot 590 CD of DDS yielded $T\frac{1}{2}$ values for the aqueous and ethanolic extracts that were significantly longer and shorter, respectively, than that of the DDS standard. At the concentration of approximately 100 μg/ml, only the ethanolic extract of Lot 590 CD exhibited a $T\frac{1}{2}$ that was not significantly longer than that of the DDS standard. At the highest concentration of approximately 170 μg/ml, the aqueous extract of Lot 111 CC exhibited a significantly longer $T\frac{1}{2}$, and the ethanolic extracts of Lots 111 CC and 590 CD exhibited significantly shorter $T\frac{1}{2}$ values compared with the $T\frac{1}{2}$ of the DDS standard.

The rate of disappearance of DDS after irradiation is clearly concentration dependent whether the starting material used is the DDS standard or aqueous or ethanolic extracts of DDS tablets. The differences in $T\frac{1}{2}$ values noted, although statistically significant, were small and do not suggest that either water- or ethanol-soluble materials from the tablets greatly influenced the susceptibility of DDS to photodecomposition by UV irradiation.

We also examined the possible influence of saturation of the aqueous solutions of DDS with oxygen or nitrogen on the rate of decay of DDS following irradiation. Aqueous DDS standards and aqueous extracts of DDS tablets were lyophilized and the residues were dissolved in air-equilibrated distilled water or in distilled water that had been gassed for 30 min with nitrogen or oxygen. Two trials were performed on each DDS preparation, and Table 14 summarizes the results obtained. As shown, small but statistically significant differences were noted for most comparisons. All tests with water saturated with nitrogen yielded $T\frac{1}{2}$ values that were significantly shorter than corresponding values for the air-equilibrated samples or those in water saturated with oxygen. Also, differences among the DDS standard and the DDS tablets were the most marked in those tests involving presaturation with nitrogen. These results suggest that the presence of oxygen in some way decreased the rate of photodecomposition of DDS in water.

Table 13

COMPARISON OF THE HALF-LIVES OF THE DDS STANDARD AND THE AQUEOUS AND ETHANOLIC EXTRACTS OF DDS TABLETS

Sample	Starting Concentration ($\mu g/ml$)[a]	$T_{\frac{1}{2}}$[b] (hr)
DDS Standard	10.3	0.37
DDS Standard	99.2	1.7
DDS Standard	172	3.8
---------- Aqueous Extract of Tablet ----------		
111 CC	10.0	0.41
111 CC	98.3	2.3[c]
111 CC	172	4.6[c]
590 CD	9.8	0.46[c]
590 CD	99.8	2.0[c]
590 CD	166	3.8
---------- Ethanolic Extract of Tablet ----------		
111 CC	9.9	0.38
111 CC	97.7	1.9[c,d]
111 CC	161	3.2[c,d]
590 CD	9.5	0.23[c,d]
590 CD	99.2	1.7
590 CD	172	3.2[c,d]

[a] Mean concentration of the respective trials in Tables 10 to 12.

[b] Half-lives were calculated using all data points from the respective trials in Tables 10 to 12.

[c] Half-life was significantly different ($P < 0.005$) from the respective half-life of the DDS Standard.

[d] Half-life was significantly different ($P < 0.001$) from the respective half-life of the aqueous extract.

Table 14

DISAPPEARANCE OF DDS IN WATER SATURATED WITH NITROGEN OR OXYGEN AFTER ULTRAVIOLET IRRADIATION

Treatment of Water	Sample or Lot of DDS	Percent of Initial DDS after Irradiation[a]					$T_{\frac{1}{2}}$[b] (hr)	Correlation Coefficient
		0 hr	0.25 hr	0.50 hr	0.75 hr	1.0 hr		
None	DDS standard	100	59.2	49.5	29.1	19.4	0.42[c]	-0.9936
	DDS standard	100	71.6	48.0	31.4	18.6		
	111 CC	100	73.9	38.6	22.7	13.6	0.35[d]	-0.9921
	111 CC	100	76.4	44.9	25.8	15.7		
	590 CD	100	65.8	40.2	18.3	12.2	0.33	-0.9897
	590 CD	100	68.8	43.8	26.2	12.5		
Saturated with nitrogen	DDS standard	100	67.3	38.6	26.7	13.9	0.36	-0.9960
	DDS standard	100	67.6	38.2	27.4	15.7		
	111 CC	100	34.9	9.6	3.6	1.2	0.16	-0.9986
	111 CC	100	41.0	11.5	3.8	1.3		
	590 CD	100	61.4	26.5	14.4	7.2	0.26	-0.9980
	590 CD	100	60.0	28.2	14.1	7.0		
Saturated with oxygen	DDS standard	100	80.9	50.0	36.4	20.0	0.44[d]	-0.9892
	DDS standard	100	72.7	54.5	36.4	20.9		
	111 CC	100	72.0	43.0	34.4	23.6	0.54	-0.9780
	111 CC	100	62.4	49.5	35.5	32.2		
	590 CD	100	68.5	50.0	37.0	16.3	0.42	-0.9782
	590 CD	100	75.3	58.0	30.9	22.2		

[a] Initial concentration was 10 μg/ml of water.

[b] Values were calculated from regression lines of the logarithmic decay of DDS using all data points from the two separate trials of each sample or lot of DDS. All comparisons of $T_{\frac{1}{2}}$ values among samples of a treatment group or among the same sample or lots of the different treatment groups were significantly different ($P < 0.01$) unless otherwise noted.

[c] Not significantly different from the $T_{\frac{1}{2}}$ for the DDS standard in water saturated with oxygen.

[d] Not significantly different from the $T_{\frac{1}{2}}$ for lot 590 CD of this treatment group.

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